“Design, Synthesis, and Biological Evaluation of Pyrazolopyrimidine Derivatives"

Thesis presented by

Noura Sayed Hanafy
B.Sc.
Faculty of Pharmacy, Misr International University
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(Pharmaceutical Chemistry)

Supervised by

Prof. Dr. Dalal Abou El Ella
Professor of Pharmaceutical Chemistry
Faculty of Pharmacy
Ain Shams University

Dr. Mohamed Kamal Abdel Hamid
Associate Professor of Pharmaceutical Organic Chemistry
Faculty of Pharmacy
Cairo University

Dr. Rabah Ahmed Taha
Associate Professor of Pharmaceutical Chemistry
Faculty of Pharmacy
Ain Shams University

Faculty of Pharmacy
Ain Shams University
2016

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List of abbreviations:

AIF Apoptosis inducing factor

Apaf-1 Apoptotic protease activating factor 1

ATP Adenosine triphosphate

BAK Bcl-2 homologous antagonist/killer

BAX Bcl-2-associated-x-protein

BR Binding region

BINAP 2,2'-Bis(diphenylphosphino)-1,1'-binaphthy

CAK CDK-activating kinase

CDKs Cyclin-dependent kinases

CVMAOS Closed-vessel microwave assisted organic synthesis

DMSO-d_6 Deuterated dimethyl sulphoxide

DTP Developmental Therapeutic Program

E_I Electron impact

ELISA Enzyme Linked Immunosorbent Assay

EtOAc Ethyl acetate

FBS Fetal bovine serum

GI_{50} Concentration of the compound causing 50% decrease in net cell growth

Het-C Heteroatom-Carbon

IAP Inhibitors of apoptosis

IC_{50} Half-maximal inhibitory concentration

kDa Kilodalton

LC_{50} Lethal concentration (Concentration of the compound causing net 50% loss of initial cells

MAOS Microwave assisted organic synthesis

MCR Multicomponent reaction

MTP Mitochondrial transmembrane potential

MWO Microwave Oven

NCI National Cancer Institute

NF-κB Nuclear Factor kappa Beta

OD Optical density

OVMAOS Open-vessel microwave assisted synthesis

PBR Phosphate-binding region

PBS Phosphate Buffered Saline

PE Petroleum ether

PKs Protein kinases

PT Permeability transition

PUMA P53 upregulated modulator of apoptosis

RPMI Roswell Park Memorial Institute medium

SD Standard deviation

SN_{2Ar} Nucleophilic substitution

SRB Sulfo-Rhodamine-B

TCA Trichloroacetic acid

TGI Total growth inhibition

TNF Tumor necrosis factor

TRAIL Tumor necrosis factor-related apoptosis inducing ligand

Tz Time zero

Abstract:

Title of thesis:

“Design, Synthesis, and Biological Evaluation of Pyrazolopyrimidine Derivatives"

Name of candidate:

Noura Sayed Hanafy

Teaching assistant of Pharmaceutical Chemistry

Heliopolis University

Thesis supervised by:

Prof. Dalal Abou El Ella

Professor of Pharmaceutical Chemistry

Faculty of pharmacy-Ain Shams University

Dr. Mohamed Kamal Abdel Hamid

Associate Professor of Pharmaceutical Organic Chemistry

Faculty of pharmacy-Cairo University

Dr.Rabah Ahmed Taha

Associate Professor of Pharmaceutical Chemistry

Faculty of pharmacy-Ain shams University

Abstract

By adopting fragmonic and structural hybrid approache, a model of structural hybrid between pyrazolopyrimidine heterocylic cores and *p*-substituted diaryl shiffs base fragment was designed. The design of small molecule was based on biologically multitarget cytotoxic lead structures like roscovitine, resveratrol to decrease drug resistance of cancer cells. The model reprsented by synthetic molecules **VIIa-t** and **IXa-i** were prepared by direct arylation of 4-chloropyrimidine derivatives Va-**f** with *p*-substituted phenolic aldehyde or *m*-substituted phenolic aldehyde either by direct nucleophilic substitution or under metal catalyzed assisted synthesis then condensation of the aldehyde products **VIa-f** and **VIIIa-f** with different aromatic amines, another model **Xa-t** was designed through alkylation of NH group of pyrazolopyrimidinone **IVa-f**. All synthetic steps were performed under open vessel microwave assisted synthesis conditions**.** Five compounds **(VIIf, VIIm, VIIo, Xj and Xr)** were selected by National Cancer Institute "NCI" (www.dtp.nci.nih.gov); under the Developmental Therapeutic Program (DTP) USA for single dose screening program at 10 uM in the full 60 cell panel. One compound **(VIIo)** was further screened at five different concentrations. All the target synthesized molecules **(VIIa-t and IXa-i)** were evaluated *in vitro* for their anti-proliferative activity against colon cancer cell line HCT-116 while synthetic molecules **Xa-t** screened against breast cancer MCF-7 at National cancer institute Egypt. Most of the synthesized compounds **VIIa-t** and **IXa-i** showed excellent antiproliferative activity ranging from 7.97 μM to 74.43 μM against HCT-116 cell line, while N-alkylated pyrazlopyrimidinone **(Xa-t)** derivatives showed a range from 8.22 and 34.78μM against MCF-7. In order to explore the mechanism of cytotoxicity, DNA-flow cytometry analysis for the effect of the molecules **(VIIo, IXi)** on HCT-116 cancer cell, which showed that these molecules arrested the cancer cells in G0/G1 phase, in addition, measurment of apoptotic regulator PUMA, BAX, caspase-3 level in colon cancer cell line after treatment with the molecule **(VIIo, IXi)** showed that they have a pro-apoptoitic activity compared with control. Also two compounds **(Xj, Xr)** from the second series were analyzed by cell cycle flow analysis where they showed cell cycle arrest of breast cancer MCF-7 mainly in Go/G1 phase. Furthermore, CDK2 kinases enzyme inhibition % assay was done for molecules **(VIIe, VIIo, VIIt, IXd, IXe, IXh, IXi)** and IC_{50} for the molecules **(IXd, IXe, IXh, IXi)** showed selective inhibition CDK2. Finally,to investigate cell cycle arrest of HCT-116 due to effect of synthetic molecule, western blot analysis for expression of CDK-2, cyclin A, P53, P21 and P27 was performed in dose dependant manner that suggested this molecule **(VIIo)** may be CDK

pathway inhibitor one of its targets. As conclusion, in this investigation, a different series of pyrazolopyrimidine derivatives were discovered as potential cytotoxic agents against HCT-16 and MCF-7 cancer cells.

The structures of final compounds were confirmed by various spectral and microanalytical data.

The study involved the synthesis of the following reported unavailable intermediates:

1] 4-Methoxyphenyl hydrazine (Ib)

2] 4-Chlorophenyl hydrazine (Ia)

3] Ethoxymethylidene malononitriles (IIa)

4] Ethoxyethylidene malononitriles (IIb)

The study involved the synthesis of the following reported unavailable intermediates under OVAMS as noval procedure to literature:

1] 5-Amino-1-phenyl-1H-pyrazole-4-carbonitrile (IIIa)

2] 5-Amino-3-methyl-1-phenyl-1H-pyrazole-4-carbonitrile (IIIb)

3] 5-Amino-1-(4-chlorophenyl)-1H-pyrazole-4-carbonitrile (IIIc)

4] 5-Amino-1-(4-chlorophenyl)-3-methyl-1H-pyrazole-4-carbonitrile (IIId)

5] 5-Amino-1-(4-methoxyphenyl)-1H-pyrazole-4-carbonitrile (IIIe)

6] 5-Amino-1-(4-methoxyphenyl)-3-methyl-1H-pyrazole-4-carbonitrile (IIIf)

7] 1-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVa)

8] 3-Methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVb)

9] 1-(4-Chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVc)

12] 1-(4-Chlorophenyl)-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVd)

13] 4-Chloro-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (Va)

14] 4-Chloro-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (Vb)

15] 4-Chloro-1-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidine (Vc)

16] 4-Chloro-1-(4-chlorophenyl)-3-methyl-1H-pyrazolo[3,4-d]pyrimidine (Vd)

In addition, the study involved the synthesis of the following novel intermediates.

1] 4-Chloro-1-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidine (Ve)

2] 4-Chloro-1-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-d] pyrimidine (Vf)

3] 1-(4-Methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVe)

4] 1-(4-Methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (IVf)

5] 4-(4-Methanoylphenoxy)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (VIa)

6] 4-(4-Methanoylphenoxy)-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (VIb)

7] 1-(4-Chlorophenyl)-4-(4-methanoylphenoxy)-1H-pyrazolo[3,4-d]pyrimidine (VIc)

8]1-(4-Chlorophenyl)-4-(4-methanoylphenoxy)-3-methyl-1H-pyrazolo[3,4-d]pyrimidine (VId)

9] 4-(4-Methanoylphenoxy)-1-(4-methoxyphenyl)-1H-pyrazolo[3,4-d] pyrimidine (VIe)

10] 4-(4-Methanoylphenoxy)-1-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-d] pyrimidine (VIf)

11] 1-(4-Chlorophenyl)-4-(3-methanoylphenoxy)-1H-pyrazolo[3,4-d] pyrimidines (VIIIa)

12]1-(4-Chlorophenyl)-4-(3-methanoylphenoxy)-3-methyl-1H-pyrazolo[3,4d]pyrimidines (VIIIb)

13] 4-(3-Methanoylphenoxy)-1-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidines (VIIIc)

14] 4-(3-Methanoylphenoxy)-1-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-d] pyrimidine (VIIId)

Also, the study involved the synthesis and characterization of the following new target compounds:

1]1-(4-Chlorophenyl)-4-[4-(phenyliminomethyl)phenoxy]-1H-pyrazolo[3,4-d]pyrimidine (VIIa)

2]4-[4-(4-Bromophenyliminomethyl)phenoxy]-1-(4-chlorophenyl)-1H-pyrazolo[3,4-d] pyrimidine (VIIb)